

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims

1. (Withdrawn) A microbial composition comprising a synergistic mixture of at least following two bacterial strains present in about equal proportion for the
 - a. *Bacillus alkalophilus*
 - b. *Bacillus sp.*
- 2.) (Currently Amended) A process for the preparation of microbial composition useful for the neutralization of alkaline textile industry waste-waters comprising:
 - (a) isolating the bacterial strains *Bacillus alkalophilus* and *Bacillus sp.* from sewage by a standard method;
 - (b) inoculating said individual bacterial strains in an Alkaline *Bacillus* broth medium containing MgSO_4 ;
 - (c) growing said individual bacterial strains for 16-24 hrs;
 - (d) inoculating said individual bacterial strains from step (c) in a modified Tryptone Soya Broth medium, having original pH value about 7.0, said pH value of the medium being adjusted to different pH values ranging between 8.0-11.0, using Tris-HCl buffer and NaOH- Na_2CO_3 buffer or NaHCO_3 - Na_2CO_3 buffer under sterile conditions;
 - (e) growing said individual bacterial strains obtained from step (d) in modified Tryptone Soya Broth medium for 16-24 hrs. at different pH values ranging from 8.0-11.0 to select the bacterial strains growing at pH 11.0 and the remaining bacterial strains unable to grow at pH 11.0 being acclimatized at pH 11.0;
 - (f) inoculating said selected acclimatized individual bacterial strains from step (e) in a modified Nutrient Broth medium, having pH values about pH 7.0, said pH being adjusted to different pH values ranging from 9.0-11.0 using NaOH- Na_2CO_3 buffer or NaHCO_3 - Na_2CO_3 buffer under sterile conditions;
 - (g) adding a dye, phenol red indicator and optionally 1% carbohydrate to said inoculated medium of individual bacterial strains obtained from step (f) to observe the change in colour for acid production and to identify the acid producing strains at pH 9.0-11.0;

(h) growing said inoculated bacterial strains obtained from step (g) for a period of at least 2 days and thereafter observing acid production by the change in colour of phenol red in said medium from red to orange, orange to yellow and by measuring the decrease in pH of said medium;

(i) selecting the acid producing bacterial strains at pH 11.0;

(j) mixing said selected bacterial strains from step (i) to obtain mixed bacterial suspension;

(k) centrifuging the mixed suspension of bacterial strains obtained from step (j) at 8,000-12,000 rpm to obtain pellet;

(l) washing the obtained pellet from step (k) by suspending the pellet in triple distilled water and re-centrifuging at 8,000-12,000 rpm; and

(m) collecting the pellet from step (l) and lyophilizing the obtained pellet to store at 1 to 4° C for longer use.

3. (Original) A process as claimed in claim 2 further comprising:

(a) reconstituting the lyophilized bacterial powder obtain in step (m) by adding 50 mL distilled water;

(b) adding 10% reconstituted bacterial strains in alkaline medium and alkaline waste-waters containing phenol red indicator and optionally 1% carbohydrate; and

(c) observing acid production by the change in colour of phenol red in modified Nutrient Broth medium and in the alkaline waste waters from red to orange, orange to yellow and by measuring the decrease in pH of the said medium as well as of alkaline waste-waters to neutral after a period of at least 2 days;

4. Cancelled

5. (Original) A process as claimed in claim 2 wherein in step (d), the medium used is Tryptone Soya Broth modified by removal of Papaic digest of soyabean meal and changing the amount of ingredients.

6. (Original) A process as claimed in claims 2 wherein in step (d), the medium used is Nutrient Both modified by addition of K_2HPO_4 and changing the amount of ingredients.
7. (Withdrawn) A method for the neutralization of alkaline waste-waters using a microbial composition, said method comprising adding a synergistic mixture of at least two bacterial strains, namely *Bacillus alkalophilus* and *Bacillus sp.* which are present in about equal proportion, with the alkaline waste-waters.
8. (Withdrawn) A method as claimed in claim 7 comprising:
- (a) reconstituting lyophilized bacterial powder obtain in step (m) of claim 2 by adding 50 mL distilled water;
 - (b) adding 10% reconstituted bacterial strains in alkaline medium and alkaline waste-waters containing phenol red indicator and optionally 1% carbohydrate; and
 - (c) observing acid production by the change in colour of phenol red in modified Nutrient Broth medium and in the alkaline waste waters from red to orange, orange to yellow and by measuring the decrease in pH of the said medium as well as of alkaline waste-waters to neutral after a period of at least 2 days;
9. (New) A process for the preparation of microbial composition useful for the neutralization of alkaline textile industry waste-waters comprising:
- (a) isolating the bacterial strains *Bacillus alkalophilus* CBTCC/Micro/8 and *Bacillus sp.* CBTCC/Micro/9 from sewage by a standard method;
 - (b) inoculating said individual bacterial strains in an Alkaline Bacillus broth medium containing $MgSO_4$;
 - (c) growing said individual bacterial strains for 16-24 hrs;
 - (d) inoculating said individual bacterial strains grown in step (c) in a modified Tryptone Soya Broth medium, having original pH value about 7.0, said pH value of the medium being adjusted to different pH values ranging between 8.0-11.0, using Tris-HCl buffer and NaOH- Na_2CO_3 buffer or $NaHCO_3$ - Na_2CO_3 buffer under sterile conditions;
 - (e) growing said individual bacterial strains obtained from step (d) in modified Tryptone Soya Broth medium for 16-24 hours at different pH values ranging from 8.0-11.0 to

select the bacterial strains growing at pH 11.0 and the remaining bacterial strains unable to grow at pH 11.0 being acclimatized at pH 11.0;

(f) inoculating said selected acclimatized individual bacterial strains from step (e) in a modified Nutrient Broth medium, having pH values about pH 7.0, said pH being adjusted to different pH values ranging from 9.0-11.0 using NaOH-Na₂CO₃ buffer or NaHCO₃-Na₂CO₃ buffer under sterile conditions;

(g) adding a dye, phenol red indicator and optionally 1% carbohydrate to said inoculated medium of individual bacterial strains obtained from step (f) to observe the change in colour for acid production and to identify the acid producing strains at pH 9.0-11.0;

(h) growing said inoculated bacterial strains obtained from step (g) for a period of at least 2 days and thereafter observing acid production by the change in colour of phenol red in said medium from red to orange, orange to yellow and by measuring the decrease in pH of said medium;

(i) selecting the acid producing bacterial strains at pH 11.0;

(j) mixing said selected bacterial strains from step (i) to obtain mixed bacterial suspension;

(k) centrifuging the mixed suspension of bacterial strains obtained from step (j) at 8,000-12,000 rpm to obtain pellet;

(l) washing the obtained pellet from step (k) by suspending the pellet in triple distilled water and re-centrifuging at 8,000-12,000 rpm; and

(m) collecting the pellet from step (l) and lyophilizing the obtained pellet to store at 1 to 4° C for longer use.

10. (New) A method for treating alkaline textile industry waste waters comprising the steps of:

(a) isolating the bacterial strains *Bacillus alkalophilus* CBTCC/Micro/8 and *Bacillus* sp. CBTCC/Micro/9 from sewage by a standard method;

(b) inoculating said individual bacterial strains in an Alkaline Bacillus broth medium

(c) growing said individual bacterial strains for 16-24 hrs;

(d) inoculating said individual bacterial strains grown in step (c) in a modified Tryptone Soya Broth medium, having original pH value about 7.0, said pH value of the medium being adjusted to different pH values ranging between 8.0-11.0, using Tris-HCl buffer and NaOH-Na₂CO₃ buffer or NaHCO₃-Na₂CO₃ buffer under sterile conditions;

(e) growing said individual bacterial strains obtained from step (d) in modified Tryptone Soya Broth medium for 16-24 hours at different pH values ranging from 8.0-11.0 to select the bacterial strains growing at pH 11.0 and the remaining bacterial strains unable to grow at pH 11.0 being acclimatized at pH 11.0;

(f) inoculating said selected acclimatized individual bacterial strains from step (e) in a modified Nutrient Broth medium, having pH values about pH 7.0, said pH being adjusted to different pH values ranging from 9.0-11.0 using NaOH-Na₂CO₃ buffer or NaHCO₃-Na₂CO₃ buffer under sterile conditions;

(g) adding a dye, phenol red indicator and optionally 1% carbohydrate to said inoculated medium of individual bacterial strains obtained from step (f) to observe the change in colour for acid production and to identify the acid producing strains at pH 9.0-11.0;

(h) growing said inoculated bacterial strains obtained from step (g) for a period of at least 2 days and thereafter observing acid production by the change in colour of phenol red in said medium from red to orange, orange to yellow and by measuring the decrease in pH of said medium;

(i) selecting the acid producing bacterial strains at pH 11.0;

(j) adding said bacterial strains selected in step (i) to alkaline textile industry waste water,

wherein said addition of said bacterial strains to alkaline textile industry waste water functions to neutralize said alkaline textile industry waste water.

11. (New) The method of claim 10, further comprising
mixing said selected bacterial strains from step (i) to obtain mixed bacterial suspension;
centrifuging the mixed suspension of bacterial strains obtained from step (j) at 8,000-12,000 rpm to obtain a pellet;

washing the pellet obtained from step (k) by suspending the pellet in triple distilled water and re-centrifuging at 8,000-12,000 rpm;

collecting the pellet from step (l) and lyophilizing the obtained pellet; and
reconstituting the pellet from step (m) in distilled water, wherein the reconstituted
pellet/distilled water mixture is added to said alkaline textile industry waste water in order to
neutralize said alkaline textile industry waste water.

12. (New) The method of claim 10, wherein said bacterial strains that are selected are
Bacillus alkalophilus CBTCC/Micro/8 and *Bacillus sp.* CBTCC/Micro/9.